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REPORT NO. 557

ELECTRON MICROSCOPIC OBSERVATIONS ON THE COLLAPSED AND DISTENDED MAMMALIAN URINARY BLADDER

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ABSTRACT

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OBJECT

The transitional epithelium of selected laboratory animals was observed with the electron microscope to obtain knowledge of its normal structure and function. This knowledge is required for an understanding of disease processes and experimentally induced changes in this tissue.

RESULTS

This study revealed some characteristics of the cell walls of transitional epithelium which could provide for the epithelial stretching present in the distended urinary bladder. There is an elaborate infolding of the two opposing cell walls in the collapsed bladder. This along with grossly visible folding of the entire epithelium could greatly increase the effective cell surface when unfolded and when the cell is flattened and elongated. Desmosomes were observed indicating that these cells are strongly attached to each other, precluding any sliding movement between cells.

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I. INTRODUCTION

The classic textbook description of transitional epithelium indicates that it consists of many cell layers in the contracted state while only two layers can be distinguished in the stretched condition (2, 4). In addition, some authors suggest that the cells rearrange themselves by sliding over one another when the bladder fills or empties and the epithelium stretches or contracts. Recently Walker (6) offered evidence that three basic cell layers exist in epithelium lining the mouse urinary bladder. This suggests that some mechanism other than sliding movement of the cells functions during stretching and contraction of transitional epithelium. Based on the results of our observations on urinary bladder epithelium of small laboratory animals, we propose a mechanism by which the epithelium can increase or decrease in surface area as necessary. This paper presents these observations and the proposed mechanism of epithelial stretching derived from them.

II. MATERIALS AND METHODS

Transitional epithelium of the urinary bladder was obtained from 9 rats, 15 mice, 2 rabbits, and 9 dogs. The rats, mice, and rabbits were anesthetized with ether and tissue from the bladder wall was removed surgically. The dogs were anesthetized with pentobarbital Na. Tissue from all animals was obtained midway between the neck and tip of the bladder. Bladders from 2 rats and 4 mice were fixed in a distended position by injecting fixative into the bladder lumen with a fine gauge needle and syringe. The fixative was allowed to remain in the bladder under pressure for 3 minutes. At this time tissue was cut from the bladder wall and placed in a pool of fresh fixative for continued fixation.

All other bladders were collapsed or only slightly distended at the time tissues were removed for fixation. The tissues were placed in a pool of fixative at ice bath temperatures within 15 seconds after removal from the body. The fixative used was osmium tetroxide buffered to pH 7.4 with veronal acetate and with 0.015 Gm of sucrose per ml added (1, 5). The specimens were dehydrated with ethanol, embedded in n-butyl and methyl methacrylate or Epon 812 (3), sectioned with a Porter Blum or Leitz Moran ultramicrotome, and examined in a RCA EMU-3 electron microscope.

III. RESULTS

Three cell layers and only three cell layers were observed in the four species when sections were cut at right angles to the bladder wall. Only when tangential sections were cut was it possible to find more than three layers. Even in these cases there were only three cell types present corresponding to the three basic epithelial layers. These three layers were similar to those described by Walker and can be seen in Figures 1 and 2 although the basement membrane is not visible in Figure 1. Walker described three layers: a distal or superficial layer of very large cells, an intermediate layer of medium sized cells, and a basal layer of small cells. Each of these cell layers consisted of a definite cell type which varied from the others not only in size but also in cytoplasmic structure.

Three cell layers were also present in those bladders which were fixed in a distended position. However, the epithelium was very thin in these cases and the cell layers were quite thin when compared to their collapsed state. The basal and intermediate layers were very thin and often were difficult to locate on low power examination. Careful study of higher power photographs always revealed three layers even though one of them often consisted of no more than two layers of cell wall with a narrow band of cytoplasm between them. This was especially true at the periphery of the cell away from the nucleus as seen in Figure 2. The extreme flattening of one or two of the layers in a stretched state probably accounts for the apparent existence of only two layers when the light microscope is utilized for observation.

An elaborate infolding of the cell walls was present in all collapsed bladders. This varied from a slight folding back and forth to a complex infolding that was impossible to trace in all of its details. The extent of the infolding or convolution of the opposing cell walls varied from one area to another and even from one end of a cell to another. The usual situation is illustrated in Figure 1 and consists of moderate folding back and forth of the membranes. In some cases the folding was so complex that a bundle of membranes existed in the area of the cell walls or even projected deep into the cytoplasm of a superficial cell. It was not always possible to trace a connection between these bundles and the obvious cell wall but both had the same structural characteristics. These masses of membranes probably correspond to the membrane tangles described by Walker, who also suggested that they were attached to the cell membranes. As seen in Figure 1 the folding was never as complex between cells of the deepest layers as it was between the superficial and intermediate cell layers.

Irregularities of the cell wall were either non-existent or slight in those bladders fixed in a distended position. The cell boundries were relatively straight lines with only an occasional fold. The extent of the slight folding varied from one area to another but it was usually most prominent between the superficial and intermediate cell layers. None of the bundles of membranes or membrane tangles seen within collapsed cells were present in cells of the distended bladder.

Although most of the cell walls in transitional epithelium were found to be flexible and irregular there were straight and rigid sections of wall separating adjacent cells of the superficial layer. This straight segment extended from the lumen surface down toward the intermediate layer for one third the depth of the surface cell in its collapsed state. The deeper half of the wall separating two superficial cells was folded as were all deeper cell membranes. These two segments, straight and folded, can be seen in the upper left of Figure 1. The straight section of the two opposing cell walls was also structurally different than the irregular folded areas. At lower powers it appeared denser and possibly wider than any other area of the cell wall. Upon examination under high magnification each cell wall of the opposing pair was often observed as a double membrane. The cell wall exposed to the bladder lumen has been described as a thickened membrane by histology books and Walker described this as a double membrane. A double membrane was also observed at the lumen surface in this study and it was often continuous with the double membrane of the straight segment described above. Thus the straight segment was composed of the two opposing cell walls, each of which was easily visible as a double membrane (Fig. 4). A double membrane was not easily observed in the deeper layers where the cell wall was convoluted and folded.

Several desmosomes were always present in the straight segment and were always present a short distance from the lumen surface (Fig. 3). The cytoplasm of the superficial cells was filled with large masses of fine filaments. These filaments were especially numerous around the desmosomes and were attached to them. The filaments were present in the deeper cell layers but were not as abundant. Desmosomes were observed between cells of all layers but they were not nearly as numerous or as well developed as those near the bladder lumen (Fig. 5).

IV. DISCUSSION

Transitional epithelium is so named because it is thought to represent a transition between stratified squamous and columnar

epithelia. It is found lining the walls of the excretory passages of the urinary system and it is subject to great mechanical changes as a result of stretching and contraction, especially in the urinary bladder. Histology textbooks indicate that it consists of many cell layers in the contracted state and two layers in the expanded state. Recently Walker offered electron microscopic evidence indicating that it always consists of three distinct layers in the mouse. These layers--superficial, intermediate, and basal, have different internal structural characteristics. Tangential sections or the thick sections utilized for light microscopy can easily create the impression of a many-layered epithelium.

The mechanical changes involved when the bladder is expanded to its fullest extent are very great and the epithelium must have the ability to stretch over wide limits. If the theory that contracted epithelium consists of many layers and these become two layers on stretching, there would have to be movement of the cells in relation to each other. This sliding of cells into a position providing two layers and flattening of these layers could provide the increase in epithelial surface necessary in an expanded bladder. However, the observations of Walker and similar observations in our laboratory indicate that three cell layers are relatively constant in the contracted and expanded state. A flattening of the cells and stretching of the cell wall could also increase the available epithelial surface but it is unlikely that the cell wall has any significant elastic properties.

The elaborate infolding or convolution of the cell wall described in this paper could provide for the increased epithelial surface necessary during distension of the urinary bladder and explain how the surface could rapidly retract following emptying and collapse of the bladder. The presence of desmosomes suggests a strong attachment of cell to cell especially near the luminal surface where they are the most numerous and the largest. This would be a special point of stress in the expanded bladder and would require a strong attachment to prevent entry of fluids into the intercellular spaces. The desmosomes along with the straight rigid section of the cell wall near the surface of the epithelium and the prominent double membrane of the cell walls probably all play a part in providing a strong cell junction. The cell walls deeper in the epithelium would have to be more flexible to permit the folding and unfolding occurring during volume change in the bladder.

The four species utilized in this study all followed the same general pattern and no attempt was made to evaluate minor differences

because of the limited number of animals examined. However, it did seem that there was more extensive folding of the cell wall in rat bladder than the other species. Changes in fluid content of the epithelial cells may also occur during stretching of the epithelium and no attempt was made to correlate cell volume with the condition of the bladder wall.

V. SUMMARY

During the course of our electron microscopic studies on the normal structure of transitional epithelium we observed some characteristics of the cell wall which could provide for the epithelial stretching present in the distended urinary bladder. We have observed an elaborate infolding of the two opposing cell walls in the collapsed bladder. This along with grossly visible folding of the entire epithelium could greatly increase the effective cell surface when unfolded and when the cell is flattened and elongated. Desmosomes were observed indicating that these cells are strongly attached to each other, precluding any sliding movement between cells.

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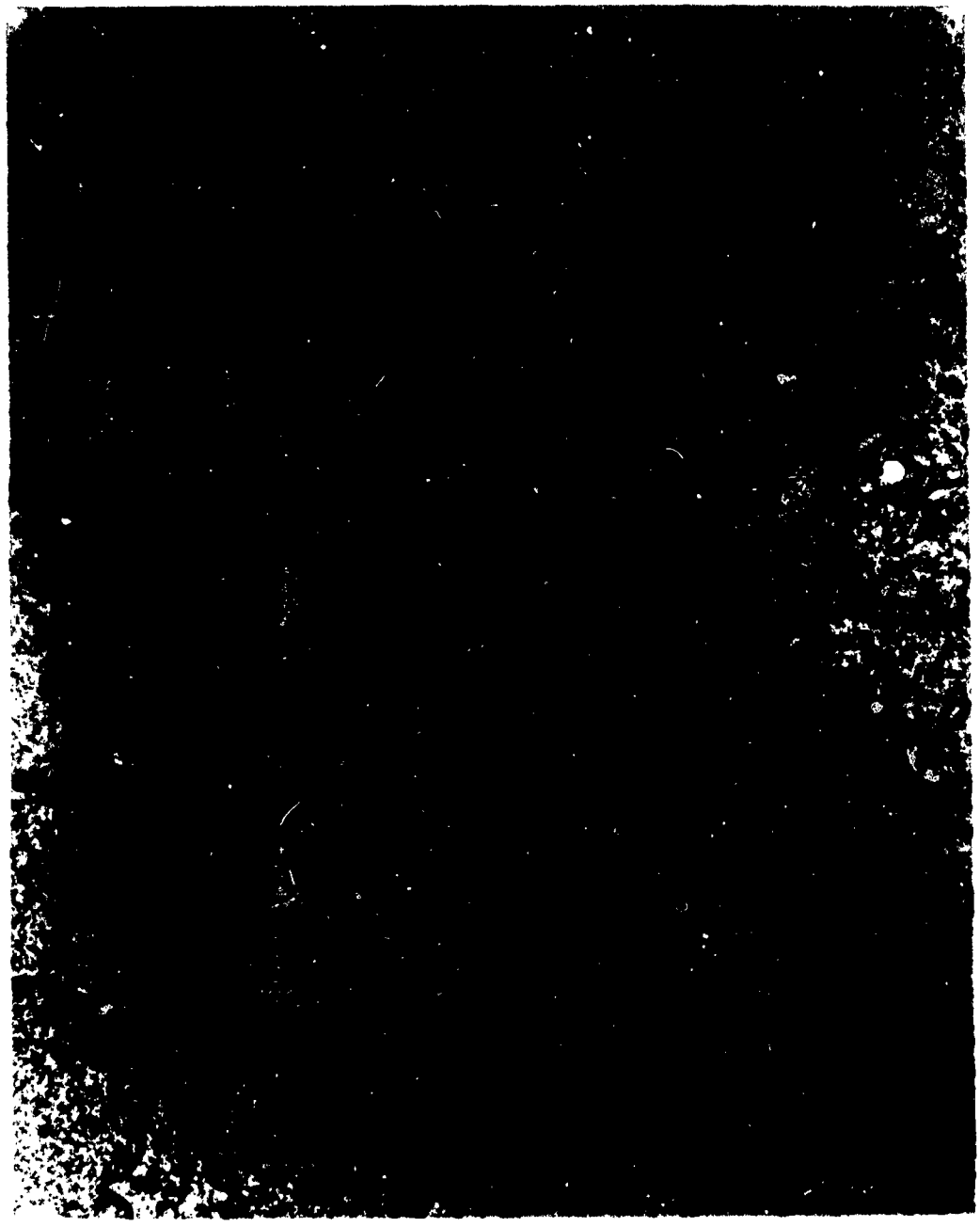

Fig. 1. Urinary bladder epithelium of the rat in a collapsed state. Note the folded cell walls and three layers of cells. X7, 200.

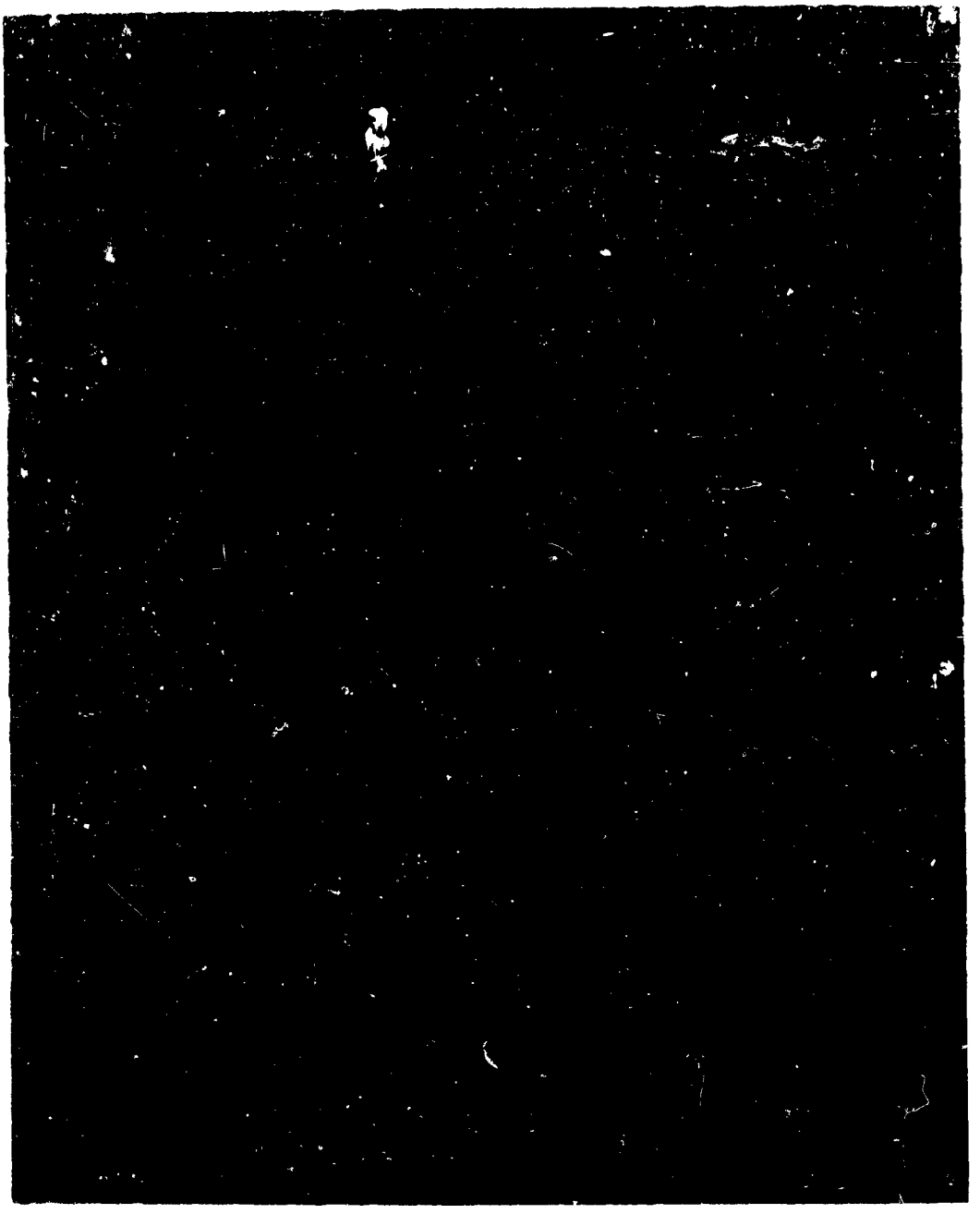

Fig. 2. Urinary bladder epithelium of the mouse fixed with the bladder distended. Note the relatively straight cell walls (arrows), three cell layers, lumen (L), and subepithelial tissue (SE). X10, 000.

Fig. 3. Junction between two superficial cells at lumen (L) surface. Note the desmosomes (D) and keratin fibers (K). X80, 000.

Fig. 4. Double membrane structure of the cell walls is readily visible at the lumen surface and in the upper one third of the walls separating two superficial cells as shown here. X300, 000.

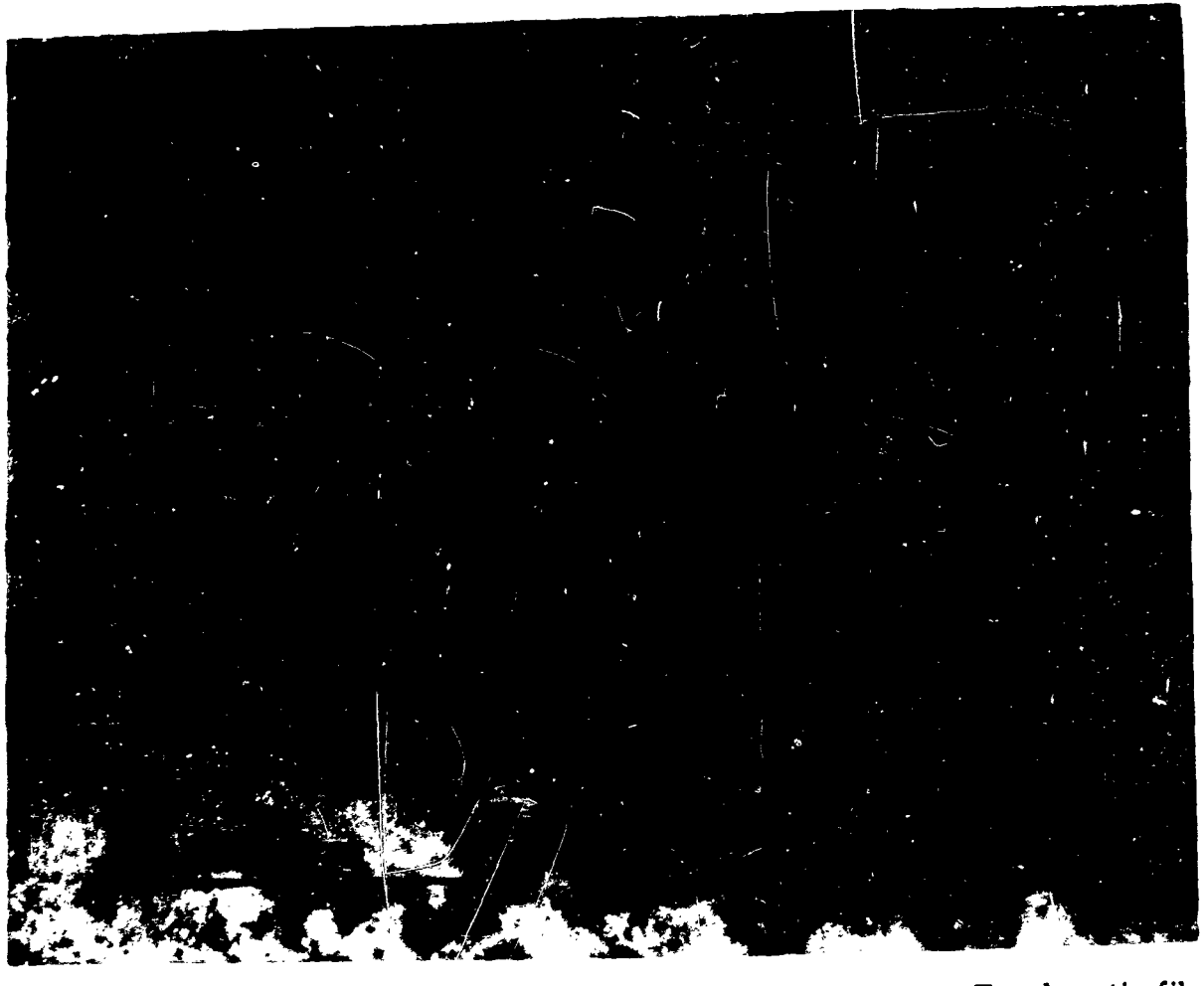

Fig. 5. Small desmosomes (D) are also found in the deeper cell layers. Few keratin fibers are present. X58, 000.

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UNCLASSIFIED
1. Transitional Epithelium
2. Electron Microscopy
3. Lab Animal Ultrastructure

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